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Magnetic field induced ordering of bile salt / phospholipid micelles: new media for NMR structural investigations

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Micelles formed from sodium glycocholate and dimyristoylphosphatidylcholine are demonstrated to form a magnetic field orientable liquid crystal within narrow ranges of composition and temperature. The utility of this medium in structural investigations of biological membrane components using deuterium NMR is discussed.

Much of our detailed knowledge of biologically important molecules has come from investigations of molecules in solution or perfectly ordered crystals. Yet, much of biological function occurs in aggregated systems like membranes or micelles where neither perfect order nor compositional homogeneity exists. Among the variety of techniques applied to membrane-associated problems [1], deuterium and phosphorus NMR have played especially important roles in improving our understanding of the biochemistry and physical chemistry of membrane components [2,3]. Here we describe a new addition to field-orientable media which allow significant simplification of NMR and other types of data on membrane constituents [4–7].

The explanation for data simplification is easily understood in the case of deuterium NMR. Deuterium, a spin one nucleus, displays both magnetic field–nuclear magnetic moment interactions and electric field gradient–nuclear quadrupole moment interactions. In a non-isotropically averaged but uniformly oriented system, the latter remove a degeneracy of transitions present when only magnetic field interactions are important. This yields two lines for each deuterated site, the splitting between the lines being dependent on the average orientation of the C-^{2}H vector with respect to the magnetic field. These splittings give essential molecular structure information. Random dispersions of bilayers, however, give rise to a superposition of pairs of lines from bilayer segments having all possible orientations in space. The resulting spectrum is called a powder pattern. Resolution of overlapping powder patterns when several deuterated sites are present, while possible, is difficult [8]. More definitive results are therefore obtained when multiple sites can be labelled and resolved in oriented rather than dispersed samples.

Ordering of biological membranes or model membranes can be achieved in several ways. One of the more common approaches uses glass plates to mechanically orient samples [9,10]. The approach, while quite generally applicable, suffers from difficulties in achieving homogeneous order and efficient use of sample volume. Seelig et al. [11] and Speyer et al. [12] have reported homogeneous orientation of certain phospholipid bilayer

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preparations without glass plates using high magnetic fields. The approach that we have used in our work also relies on magnetic field induced ordering but uses lyotropic liquid crystals in the nematic phase which order more reproducibly and at lower fields [13]. The system that we used initially is a potassium laurate system developed by Forrest and Reeves [14]. Deuterium NMR of this system shows a clean pair of lines from each distinct C-^{2}H bond [15,16]. Despite the spectroscopic advantages, the potassium laurate system, and many other field-ordered liquid crystals, are not ideal models for membrane preparations. They have a hydrophobic/aqueous interface, but the carboxylate headgroups are more highly charged than the phospholipid headgroups, and interfacial geometry can be quite different. Also, the high pH of these preparations (about 9) could cause complications when used as solvents for biomolecules like glycoproteins and glycolipids. It is therefore of considerable importance to develop other field-ordered systems which more closely model biological membranes.

The phenomenon of ordering of liquid-crystalline microdomains has been investigated quite thoroughly [17] and the requisites are fairly well understood. First, the system has to be magnetically anisotropic. This is rarely a problem since most molecules have an inherent diamagnetic anisotropy. In a magnetic field the molecule develops an orientation dependent magnetic moment that interacts with the field producing a torque which tends to align the molecule consistent with the minimum free energy orientation. For a single molecule in currently accessible fields, this torque is very small and cannot compete with thermal disorder. Liquid crystals, however, show cooperativity, allowing the net torque to override thermal motion and bring about the alignment of all individual molecules or micellar units. Second, the system must be sufficiently mobile to order on time scales acceptable to laboratory operations. At high viscosities, the rates of reordering required to achieve the minimum energy configuration are prohibitively small while at very low viscosities insufficient cooperativity may exist. These requirements are met in the potassium laurate system through an appropriate balance of particle geometry and particle spacing. The potassium laurate system is believed to be composed of micelles 80 Å long and 40 Å in diameter packed at a density that would give micelle-micelle spacing of 90 Å [14]. Potassium laurate/decanol systems are believed to be discoidal particles with similar sizes and spacings. Both exhibit high levels of cooperativity with moderate viscosity.

The question that arises is whether other micellar systems of more direct biological interest or more closely related to lipid membranes would order as successfully if they had similar geometries and packed similarly. Bile salt-phosphatidylcholine micelles, important in lipid metabolism, have been extensively studied [18–21]. Studies on dilute solutions, using X-ray and quasi-elastic scattering techniques, have determined the average dimensions of the micelles, their shape, and the dependence of these properties on bile salt/phosphatidylcholine molar ratios [19]. At bile salt rich ratios, centrosymmetric spherical micelles have been characterized, but for phosphatidylcholine rich ratios (less than 2 : 1 bile salt/phosphatidylcholine), disk shaped bilayers of 50 Å thickness and diameters around 500 Å have been characterized [22]. The latter would seem to have properties suitable for the production of ordered phases.

Here we demonstrate field induced order in a bile salt system. The system is a mixture of sodium glycocholate and dimyristoylphosphatidylcholine (DMPC). Based on requirements for field ordered micelles, we will work largely above molar ratios of 2 : 1 (DMPC/bile salt) where micellar dimensions and shape are similar to established systems and choose water concentrations similar to the potassium laurate system (≈ 60%) as a starting point. We will monitor the deuterium spectra of H^{2}HO in rapid exchange with molecules associated with the micellar surface in order to detect field induced order in the system. Although we have also used small amounts of micelle soluble deuterium labelled fatty acids and glycolipids to confirm field ordering, splitting of the H^{2}HO resonance provides an efficient and sensitive monitor.

Sodium glycocholate and DMPC were obtained from Sigma Co., St. Louis, MO, and used without further purification. ^{2}H$_2$O was obtained from Aldrich Chemicals, Milwaukee, WI. Following the procedure outlined by Small et al. [21], sodium glycocholate and DMPC were weighed out in the correct proportions and dissolved in a small volume of ethanol. This solution was frozen and dried in vacuo for 48 h. The dried mixture was transferred to 5-mm NMR tubes and the deuterium enriched buffer (0.15 M NaCl/Tris, pH 8) was added in aliquots until the water content reached 60–65%. The sample was heated, vortexed and centrifuged at each addition of buffer and allowed to equilibrate at 40–45 ° C for at least 24 h before the next addition of buffer. The following molar ratios (DMPC/bile salt) were examined: 2 : 1, 3 : 1, 4 : 1 and 8 : 1 in the concentration range 60–65% (w/v) lipid/buffer. The bile salt-DMPC mixtures form clear liquid crystalline mesophases within these limits. With higher contents phase separation sets in.

Deuterium spectra of these phases were acquired on a homebuilt 11.5 T spectrometer operating at 75 MHz for deuterium. The samples were heated to 40 ° C and allowed to cool down in the magnetic field to a temperature above the expected gel to liquid crystal transition temperature (30 ° C). Equilibration for at least 30 min prior to acquisition was allowed. Spectra were acquired in quadrature pulse Fourier transform mode with repetition rates of 0.16 s. Each scan was acquired with 25 kHz sweepwidth, in 8K time domain points, and was included in an average of approximately 3000 scans. Processing included exponential multiplication (5 Hz linebroadening) before Fourier transformation.

We expect the percentage of water to be the decisive factor in achieving an ordered phase. Samples are very viscous and probably have extended structures at low water concentrations (less than 58%) that would not allow reordering in the timespan of the typical experiment. At high water concentrations, discoidal micelles are probably broken into smaller isotropically tumbling units that again do not order successfully. Fig. 1 illustrates the effect of an increase in water concentration for a DMPC/sodium glycocholate 2 : 1 system. It shows a transition from the powder pattern expected for random dispersions of large particles at lower water concentrations to an isotropic singlet, expected for isotropically tumbling micelles formed at higher water concentrations. At intermediate points simple two line spectra characteristic of liquid crystalline order are seen. Ordering of discoidal systems can occur with normals to the disk surface being either parallel or perpendicular to the applied field [14]. In the present case, observation of a factor of two reduction in the splitting after a 90 ° rotation of the sample about an axis perpendicular to the field suggests a parallel orientation. Parallel orientations prove advantageous in that rapid rotation about the normal of molecules anchored to the surface is not required to provide well resolved spectra.

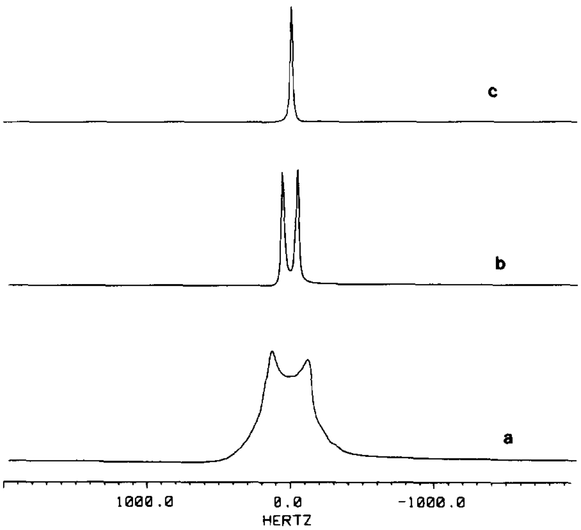

Fig. 1. ^{2}H-NMR spectra of H^{2}HO in a 4:1 DMPC/sodium glycocholate system at 303 K: (a) 43% water; (b) 63% water; (c) 73% water.

The DMPC/ glycocholate system would be an even more useful model of a membrane fragment if the percentage of DMPC could be increased. At higher phosphatidylcholine content the disks approach lamellar structures as they become larger and have higher percentages of DMPC on the discoidal surfaces. One might expect higher cooperativity and more complete order in these larger systems as long as the viscosity remained low enough. Systems with molar ratios lower than 2 : 1 did not order due either to the small size or to the near spherical symmetry of the micelles. The other systems, 2 : 1, 3 : 1, 4 : 1, and 8 : 1, all showed almost complete field-induced order. At phos-

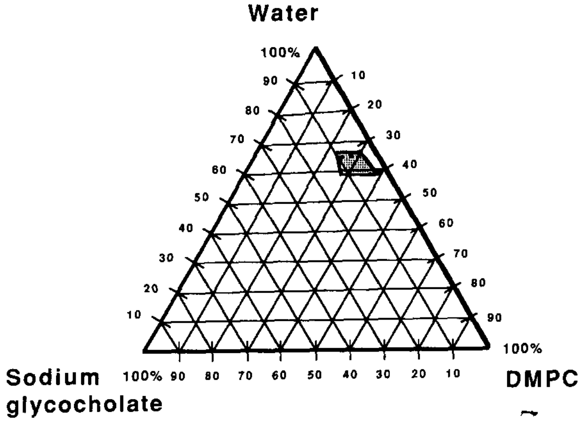

Fig. 2. Phase diagram for the DMPC/sodium glycocholate/ water system at 303 K, showing the field ordered region.

phatidylcholine concentrations higher than this, phase separation took place before an ordered phase could be observed. Results of compositional studies are summarized in the partial phase diagram of Fig. 2.

Figs. 3, 4 and 5 demonstrate the effect of temperature on the 2 : 1, 4 : 1, and 8 : 1 systems. Note that in each case raising the temperature leads to larger splittings. The temperature trend is contrary to the notion that greater order produces larger splittings, but remember that the H^2HO splittings are a complex function of average geometry of surface associated water, ratios of surface to bulk water, and a micellar order parameter. All of these parameters change with temperature. In particular surface area expansion at higher temperatures may lead to an increased fraction of surface associated water and hence larger splittings. At high temperatures, an isotropic component appears and grows at the expense of the ordered doublet. The temperature at which the isotropic singlet appears varies as a function of both phosphatidylcholine and water content–the lower the water content and the higher the phosphatidylcholine content, the higher this temperature.

Field-induced ordering of bile salt micelles is not restrict to sodium glycocholate/DMPC micelles. This phenomenon appears to be fairly widespread and was also observed with a dihy-

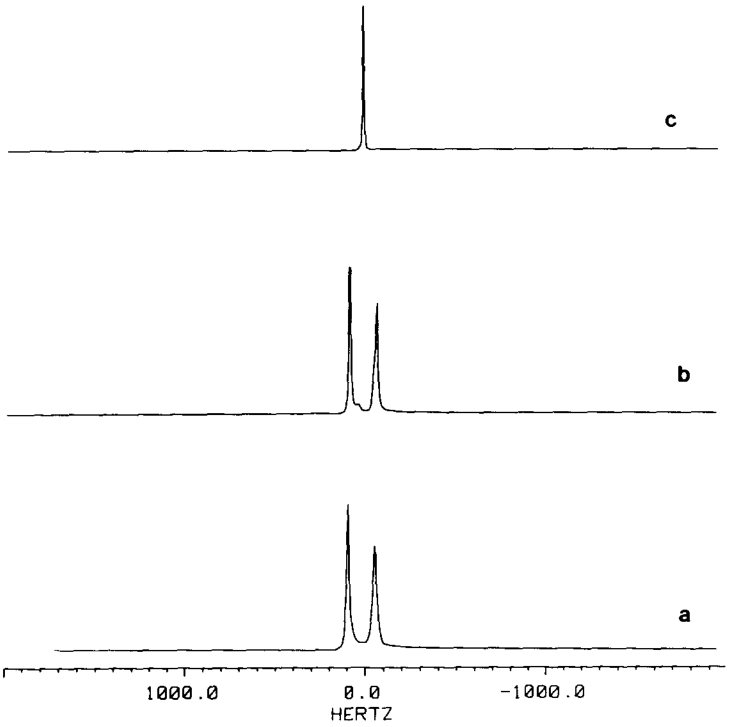

Fig. 3. Effect of temperature on 2 : 1 DMPC/sodium glycocholate system (64% water): (a) 303 K; (b) 313 K; (c) 323 K.

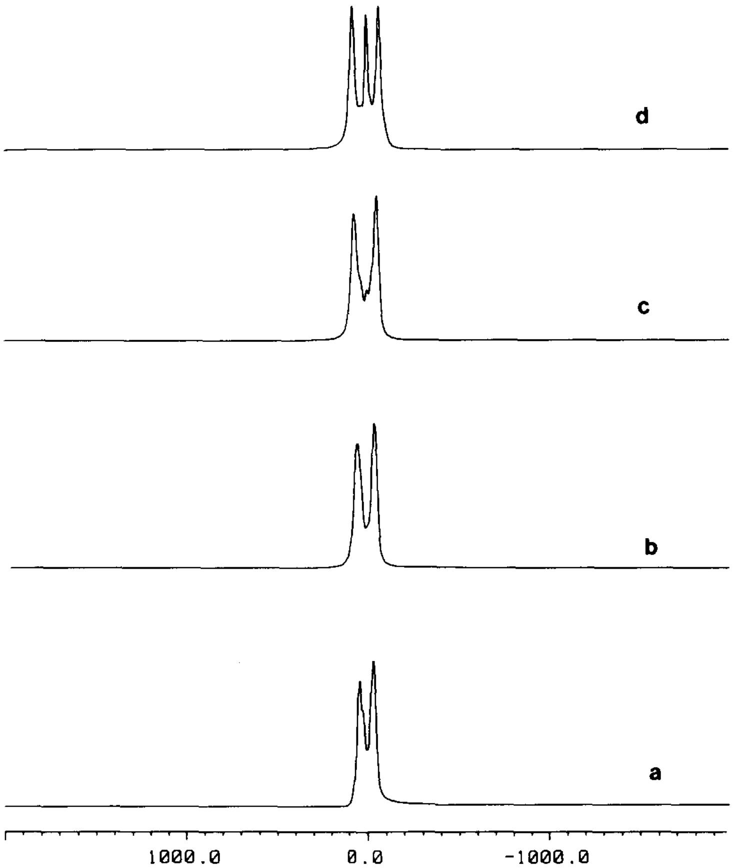

Fig. 4. Effect of temperature on 4 : 1 DMPC/sodium glycocholate system (64% water): (a) 303 K; (b) 313 K; (c) 323 K; (d) 333 K.

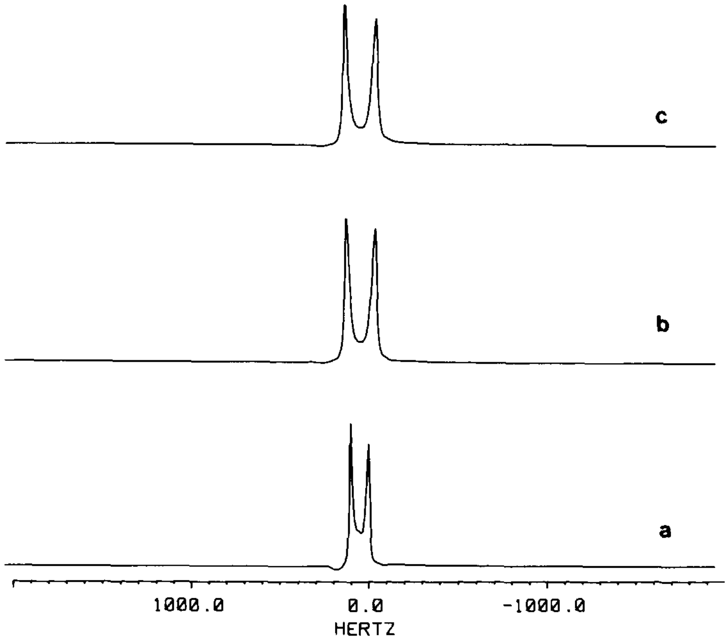

Fig. 5. Effect of temperature on 8 : 1 DMPC/sodium glycocholate system (64% water): (a) 303 K; (b) 313 K; (c) 323 K.

droxy bile salt, sodium glycodeoxycholate. Micellar systems composed of DMPC and conjugate salts of taurine, sodium taurocholate, for example, also demonstrated field induced ordering.

We have thus demonstrated field induced ordering in several bile salt-DMPC micellar systems in the composition range 60–67% water, 30–25% DMPC and 10–5% bile salt/DMPC. In the limit of high DMPC content, these systems are reasonable models for biological membranes since they form large discoidal micelles, with surfaces rich in phospholipids. Field-induced ordering of these micellar phases is expected to result in a considerable simplification of NMR spectra of molecules anchored in the phospholipid matrix such as deuterated glycolipids, important in membrane surface receptor interactions.

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